Mechanism of protein protection by desiccation-tolerance molecules

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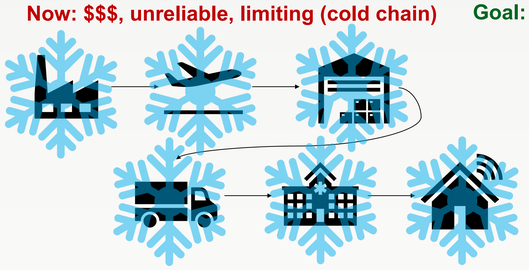
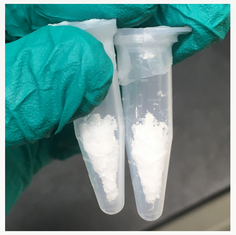
Background and Significance

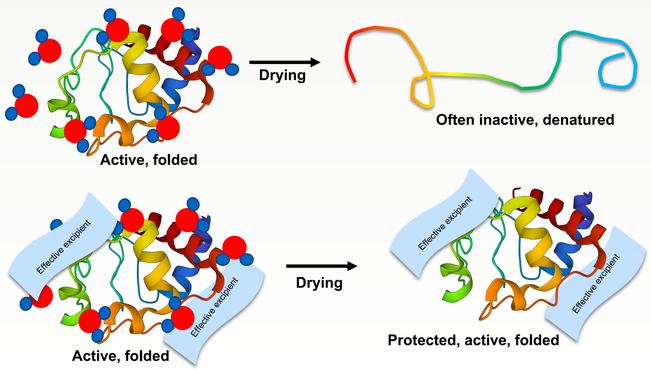
Now: $$$, unreliable, limiting (cold chain)



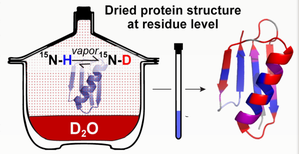
Goal: cheap, easy, accessible (drying)



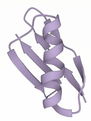
- Lifesaving protein-based drugs in solution require costly refrigeration, impeding accessibility[1,2]
- Drying increases stability and shelf life and is relatively cheap and easy, **but most proteins cannot withstand dehydration[3]**
- Protective molecules called excipients are added to safeguard proteins during drying[4,5]
- Excipient formulation empirical and of varying efficacy[4,5]
- **Lack of high-resolution information about dry proteins;[6] we do not understand protection mechanisms**



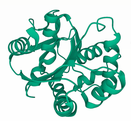
- We developed Liquid-Observed Vapor Exchange NMR **(LOVE NMR) to study dry protein structure at the residue level[7,8]**
- Understanding dehydration protection will allow rational design of excipient formulations, making protein products more affordable and accessible



Model proteins studied with LOVE NMR



GB1
PDB 1GB1

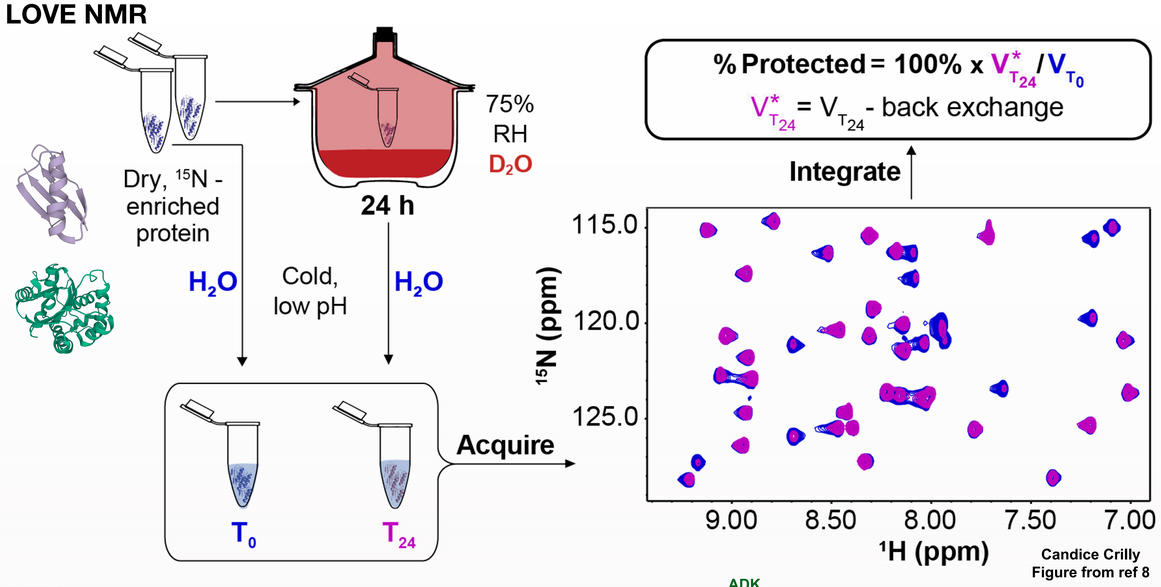


Adenylate kinase (ADK)
PDB 1AKE

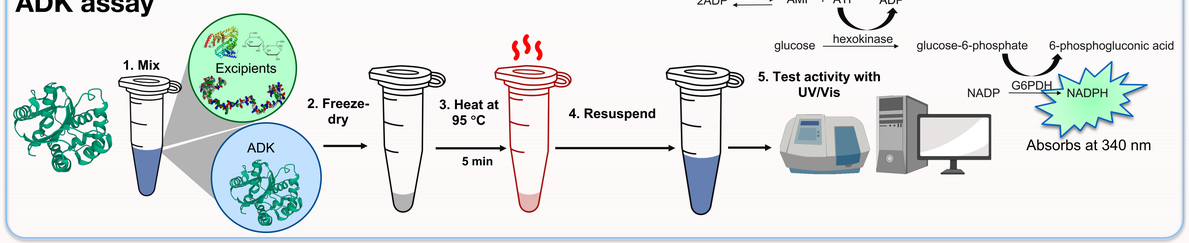
Allows observation of structure and function

Methods

LOVE NMR

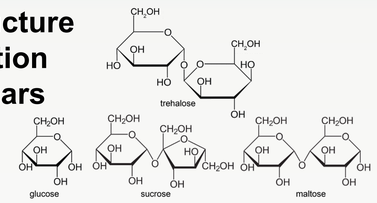


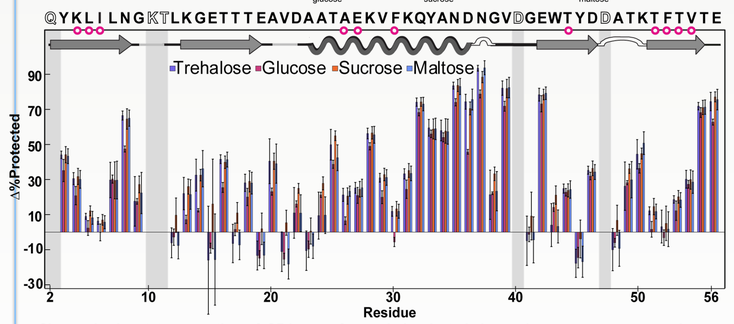
ADK assay



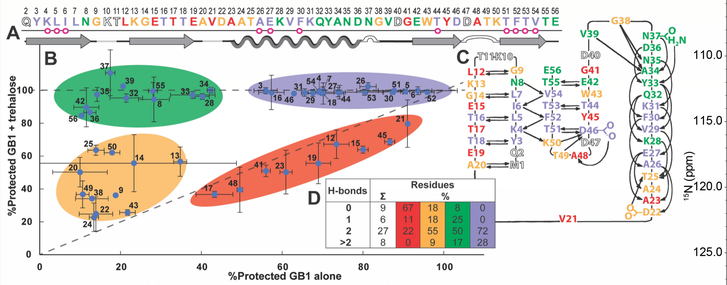
Results

GB1 structure protection by sugars



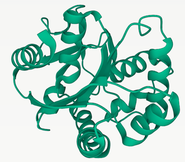


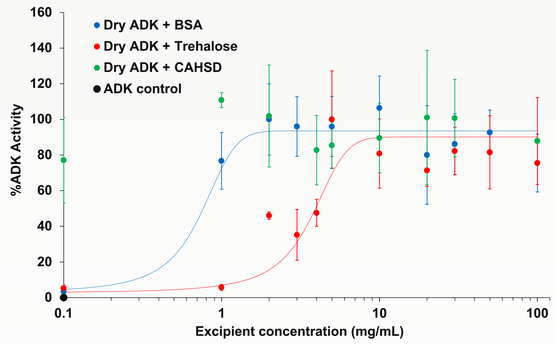
Change in dry-state protection of GB1 upon freeze-drying in trehalose, glucose, sucrose, or maltose. Δ%Protected = %Protected$_{sugar}$ – %Protected$_{-sugar}$. The primary and secondary structure of GB1 (PDB 2QMT) are shown at the top. Magenta circles indicate solution global-unfolding residues. Shaded boxes and open letters indicate missing data from rapid back exchange. Error bars represent standard deviations propagated from triplicate analysis.



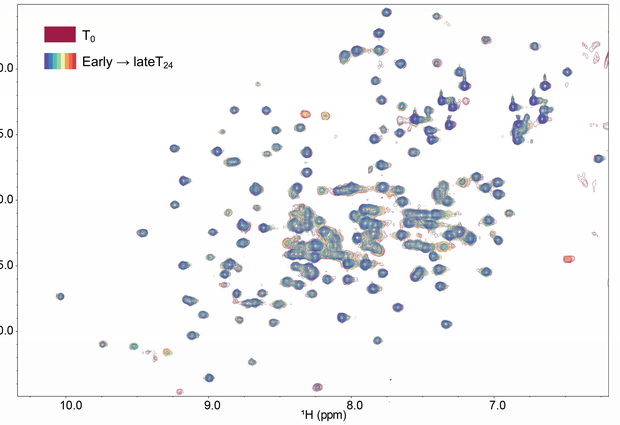
Dry state protection of GB1 in the presence and absence of trehalose. A) Residues are color-coded by their position in panel B. **B)** %Protected GB1 + trehalose versus %Protected GB1 alone. Dashed lines on the diagonal and at 100% protection in trehalose are of no theoretical significance. Error bars represent standard deviations from triplicate analysis. Shaded ellipses group the data (red, trehalose decreases protection; orange, low protection of GB1 alone and moderate trehalose protection; green, low protection in GB1 alone but ~100% protection in trehalose; purple, high protection in GB1 alone but ~100% in trehalose). **C)** Groupings mapped onto the H-bonding in GB1. Adapted with permission from ref (**9**). Copyright 1991 American Association for the Advancement of Science. **D)** H bonds and protection. **Σ** indicates the number of residues with 0, 1, 2, or >2 intramolecular H-bonds from data in panel C. Colored columns show percent of residues from each group with 0, 1, 2, or >2 intramolecular H-bonds from panel C .

ADK activity and structure protection



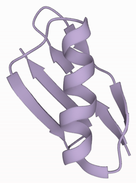


Protection of ADK activity against desiccation-induced inactivation. ADK (10 g/L) was mixed with additives, desiccated, then rehydrated with 50 mM Tris-HCl pH 7.4, 50 mM KCl. Percent activity was determined by comparison to the same solution stored at 4 °C. Error bars represent standard deviations from triplicate analysis. ADK control is with no excipient. Curves are a visual guide but have no theoretical significance.

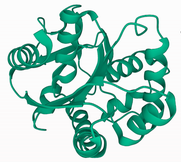


LOVE NMR with ADK. Overlaid HSQC spectra from T_0 and serial T_{24} (40 mM MOPS, 50 mM NaCl, pH 6.7, 298.2 K).

Conclusions



- Protection not unique to trehalose
- Sugars stabilize intra-protein H-bonds
- Water replacement plays a role in protection by sugars
- Trehalose likely prevalent in nature due to its covalent stability



- LOVE NMR, activity assay, structure and function protection assessment achievable

Check out our recent Protein Science paper where we use LOVE NMR to understand protection by desiccation-tolerance proteins!

References and Acknowledgments

We thank Candice Crilly for pioneering and naming LOVE NMR, Stu Parham for spectrometer maintenance and advice, and the Pielak Lab for helpful discussion. This work was supported by NIH grant R01GM127291 (GJP).

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